

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant: Kubek

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Commissioner for Patents
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DECLARATION UNDER 37 C.F.R. § 1.132 OF MICHAEL J. KUBEK

I, Michael J. Kubek, do hereby declare as follows:

1. I received a Bachelor of Science degree in Biology in 1967 from the University of Dayton, a Master of Science degree in Endocrinology in 1969 from the University of Dayton, and a Doctor of Philosophy in Neuroendocrinology in 1975 from the University of Rochester School of Medicine. Following my graduate work, I was employed as a postdoctoral fellow in the Center for Endocrinology, Metabolism and Nutrition at Northwestern University School of Medicine from 1975 - 1978.

2. I have been employed as a Professor in the Indiana University School of Medicine from 1978 to the present. Since 1987, I have been an Associate Professor of Neurobiology and an Associate Professor of Anatomy and Cell Biology in the Indiana University School of Medicine. Prior to my current position, I was an Assistant Professor of Anatomy and Neurobiology from 1982 - 1987, and an Assistant Professor of Anatomy from 1978 - 1982 in the Indiana University School of Medicine.

3. I am the author or co-author of over 40 peer-reviewed research papers, as well as several book chapters, in the fields of neurobiology, neuroendocrinology, and nanotechnology, as set forth in the attached Biographical Sketch.

4. I am the named inventor in the present application. The present invention relates to a method for selectively increasing glutamate and/or aspartate release in a central nervous system locus in a site-specific manner comprising the steps of: selecting a central nervous system locus; and providing prolonged release of thyrotropin-releasing hormone *in situ* at the central nervous system locus over a period of time by placing at least one biodegradable, non-bursting, non-spherical microstructure into the central nervous system locus, wherein the microstructure comprises 1 - 90% thyrotropin-releasing hormone and the remainder a biodegradable matrix.

5. Given that biodegradable drug-releasing implants are inserted into specific loci in the brain to deliver local drug therapy, maintaining the location of the implant throughout the entire degradation cycle is important to maintain effective therapy. Assuming that constructs of all shapes and sizes degrade at a uniform rate proportionate to their surface area, a spherical implant of any size will shrink from its original size to a size where its largest cross section is not of sufficient size to prevent construct migration through the intercellular space. This property enables spherical implants to migrate away from the intended therapeutic region during degradation.

6. Unlike a spherical construct, a non-spherical construct with a length greater than its width, such as a cylinder, microdisk, or a rod, would be able to maintain a one dimensional length of sufficient size throughout the entire degradation process, thereby preventing migration during construct degradation. While both spherical and non-spherical constructs degrade at a constant rate proportionate to their surface area, only the non-spherical construct can maintain a sufficiently large one-dimensional length to prevent diffusion of the implant over the entire degradation of the construct, enabling the therapy provider to more closely control the location of drug delivery.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so

made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

A handwritten signature in black ink, appearing to read "Michael J. Kubek", written over a horizontal line.

Date: December 10, 2007

Michael J. Kubek, Ph.D.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Michael J. Kubek	POSITION TITLE Associate Professor of Neurobiology		
eRA COMMONS USER NAME mjkubek			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
University of Dayton, Dayton, OH	B.S.	1967	Biology
University of Dayton, Dayton, OH	M.S.	1969	Endocrinology
University of Rochester Sch. Med., Rochester,	Ph.D.	1975	Neuroendocrinology
Northwestern University Sch. Med., Chicago, IL	Postdoc	1975-1978	Neuroendocrinology

A. Positions and Honors.

POSITIONS AND EMPLOYMENT

1967-1969 Teaching Assistant, Department of Biology, University of Dayton
1970-1975 Predoctoral Fellow, Depts. of Anatomy and Medicine, U. Rochester School of Medicine
1975-1978 Postdoctoral Fellow, Center for Endocrinology, Metabolism and Nutrition,
Northwestern University School of Medicine
1978-1982 Assistant Professor of Anatomy, Department of Anatomy, Indiana University School
of Medicine
1982-1987 Assistant Professor of Anatomy and Neurobiology, Depts. of Anatomy and Psychiatry,
Indiana University School of Medicine
1987-Present Associate Professor of Anatomy & Cell Biology and Neurobiology, Depts. of Anatomy
and Psychiatry, Indiana University School of Medicine
Military Service
Captain, U.S. Army Reserve, Chemical Corps, 1974-80.
Mobilization Designee: U.S. Army Research Institute of Chemical Defense, Edgewood, MD.
Laboratory of Dr. Ludwig A. Sternberger, Chief: Immunology Branch, Biochemical Research
Laboratory, Edgewood, MD., 1975-77.
Laboratory of Dr. James L. Meyerhoff, Chief Neuroendocrinology and Neurochemistry Branch,
Department of Medical Neuroscience, Walter Reed Army Institute of Research, Washington,
D.C., 1978-80.

HONORS AND AWARDS

Alpha Epsilon Delta Honor Society, University of Dayton
Recipient, NIH Predoctoral Fellowship, University of Rochester School of Medicine
Recipient, NIH Postdoctoral Traineeship, Northwestern University School of Medicine
Recipient, US Patents, 6,303,134 (2001), 6,491,939 (2002), 6,699,491 (2004), 7,229,635B2 (2007)
Pharmacotherapeutic Process and Composition for CNS Disorders.

B. Selected peer-reviewed publications (in chronological order).

Kubek, M.J., Lorincz, M.A. and Wilber, J.F.: The identification of thyrotropin-releasing hormone (TRH) in
hypothalamic and extrahypothalamic loci of the human nervous system. Brain Res. 126: 196-200, 1977.
Kubek, M.J., Wilber, J.F. and George, J.M.: The distribution and concentration of thyrotropin-releasing
hormone in discrete human hypothalamic nuclei. Endocrinol. 105:537-540, 1979.
Kubek, M.J., Lorincz, M.A. and Wilber, J.F.: Localization of gonadotropin releasing hormone (GnRH) in
hypothalamic and extrahypothalamic loci of the human central nervous system. Horm. Metab. Res. 11:25-
29, 1979.

- Kubek, M.J. and Wilber, J.F.: Leucine-Enkephalin: Identification and distribution in hypothalamic and extrahypothalamic loci of the human nervous system (CNS). *Neurosci. Lett.* 18:155-161, 1980.
- Childs, G.V., Yang, H., Tobin, R.B., Wilber, J.F. and Kubek, M.J.: Effects of thyroidectomy, propylthiouracil, and thyroxine on pituitary content and immunocytochemical staining of thyrotropin (TSH) and thyrotropin releasing hormone (TRH). *J. Histochem. Cytochem.* 29:357-363, 1981.
- Kubek, M.J., Rea, M.A., Hodes, Z.I. and Aprison, M.H.: Quantitation and characterization of thyrotropin-releasing hormone in vagal nuclei and other regions of the medulla oblongata of the rat. *J. Neurochem.* 40:1307-1313, 1983.
- Kubek, M.J. and Sattin, A.: Effect of electroconvulsive shock on the content of thyrotropin-releasing hormone in rat brain. *Life Sci.* 34:1149-1152, 1984.
- Kubek, M.J., Meyerhoff, J.L., Hill, G.T., Norton, J.A. and Sattin, A.: Effects of subconvulsive and repeated electroconvulsive shock on thyrotropin-releasing hormone in rat brain. *Life Sci.* 36:315-320, 1985.
- Kubek, M.J.: Thyrotropin-releasing hormone: localization of specific hypothalamic and extrahypothalamic sites of CNS modulation. In: *Neuroregulation of Autonomic, Endocrine and Immune Systems*, R.C.A. Frederickson, H. Hendrie, J.N. Hingtgen, M.H. Aprison (Eds.), Martinus-Nijhoff, Boston. pp. 265-301, 1986.
- Faden, A.I., Hill, T.G., and Kubek, M.J.: Changes in TRH immunoreactivity in spinal cord after experimental spinal injury. *Neuropeptides* 7:11-18, 1986.
- Sattin, A., Hill, T.G., Meyerhoff, J.L., Norton, J.A. and Kubek, M.J.: The prolonged increase in thyrotropin-releasing hormone in rat limbic forebrain regions following electroconvulsive shock. *Reg. Peptides* 19:13-22, 1987.
- Kubek, M.J., Low, W.C., Sattin, S., Morzorati, S.L., Meyerhoff, J.L. and Larsen, S.H.: Role of TRH in Seizure Modulation. *Ann. N.Y. Acad. Sci.* 553:286-303, 1989.
- Low, W.C., Farber, S.D., Hill, T.G., Sattin, A. and Kubek, M.J.: Evidence for Extrinsic and Intrinsic Sources of Thyrotropin-Releasing Hormone (TRH) in the Hippocampal Formation as Determined by Radioimmunoassay and Immunocytochemistry. *Ann. N.Y. Acad. Sci.* 553:574-578, 1989.
- Low, W.C., Roepke, J., Farber, S.D., Hill, T.G., Sattin, A. and Kubek, M.J.: Distribution of thyrotropin-releasing hormone (TRH) in the hippocampal formation as determined by radioimmunoassay. *Neurosci. Lett.* 103:314-319, 1989.
- Meyerhoff, J.L., Sates, V.E. and Kubek, M.J.: Elevated TRH in pyriform cortex after partial and fully-generalized kindled seizures. *Brain Research* 525:144-148, 1990.
- Shapiro, S., Kubek, M.J., Sanders, S., Durbin, S., Goodwin, S. and Javed, T.: Regional changes in central nervous system thyrotropin-releasing hormone after pentylenetetrazol (PTZ) induced seizures in dogs. *Neurosurgery* 31:935-939, 1992.
- Sattin, A., Kubek, M.J., Low, W.C., Staly, C.J. and Simon, J.R.: Some regional anatomical relationships of TRH to 5-HT in rat limbic forebrain. *Neurochem. Res.* 17:469-474, 1992.
- Kubek, M.J., Knoblach, S.M., Sharif, N.A., Burt, D., Buterbaugh, G.G. and Fuson, K.S.: TRH gene expression and receptors are differentially modified in limbic foci by seizures. *Ann. Neurol.* 33:70-76, 1993.
- Morzorati, S.L. and Kubek, M.J.: Septal TRH in alcohol-naive P and NP rats and following alcohol challenge. *Brain Res. Bull.* 31 :301-304, 1993.
- Morzorati, S.L. and Kubek, M.J.: TRH antagonism of ethanol sedation in alcohol-preferring and-nonpreferring rats. *Neuropeptides*, 25:283-287, 1993.
- Knoblach, S.M. and Kubek, M.J.: Thyrotropin-releasing hormone release is enhanced in hippocampal slices after electroconvulsive shock. *J. Neurochem.* 62:119-125, 1994.
- Shapiro, S., Kubek, M.J., Seimers, E., Daly, E., Callahan, J. and Putty, T.: Quantification of thyrotropin-releasing hormone changes and serotonin content changes following graded spinal cord injury. *J. Surgical Res.* 59(3): 393-398, 1995.
- Knoblach, S. and Kubek, M.J.: Increases in thyrotropin-releasing hormone (TRH) mRNA expression induced by a model of human temporal lobe epilepsy: Effect of partial and complete kindling. *Neuroscience* 76(1): 85-95, 1997.
- Knoblach, S. and Kubek, M.J.: Changes in TRH levels in hippocampal subregions induced by a model of temporal lobe epilepsy: Effect of partial and complete kindling. *Neuroscience* 76 (1): 97-104, 1997.
- Kubek, M.J., Shih, T.M. and Meyerhoff, J.L.: Thyrotropin-Releasing Hormone (TRH) is markedly increased in the rat CNS following Soman-induced seizures. *Brain Research* 747: 328-331, 1997.

Kubek, M.J., Liang, D., Byrd, K.E. and Domb, A.J.: Prolonged seizure suppression by a single implantable polymeric- TRH microdisk preparation. *Brain Res.* 809:189-198, 1998.

Kubek, M.J., Ringel, I., and Domb, A.J.: Issues related to intranasal delivery of neuropeptides to temporal lobe targets. *The Blood Brain Barrier, Drug Delivery and Brain Pathology*, pp. 323-350, 2001.

Domb, A.J. and Kubek, M.J., Synthesis of Poly (corboxyphenoxypropane-sebacic anhydride) for the delivery of drugs to the brain. *The Blood Brain Barrier, Drug Delivery and Brain Pathology*, pp. 351-362, 2001.

Kubek, M.J. and Garg, B.: Thyrotropin-Releasing Hormone in the Treatment of Intractable Epilepsy. *Pediatric Neurology* 26: 9-17, 2002.

Yard, M., Lahiri, D. K., Ringel, I., Domb, A. V., and Kubek, M. J.: Thyrotropin-Releasing Hormone (TRH; Protirelin) Affects Levels of G α Protein in the Superfused Rat Hippocampal Dentate Gyrus. *Epilepsia* 44 (Supp. 9):191, 2003.

Nie, Y., Schoepp, D. D., Klaunig, J.E., Yard, M., Lahiri, D.K. and Kubek, M.J.: Thyrotropin-releasing Hormone (Protirelin) inhibits potassium-stimulated glutamate and aspartate release from hippocampal slices *in vitro*, *Brain Research* 1054: 45-54, 2005

Joshi, M.A., Jeoung, N.H., Obayashi, M., Hattab, E.M., Bracken, E.G., Liechty, E., Kubek, M.J., Vatter, K.M., Wek, R.C. and Harris, R.A.: Impaired Growth and Neurological Abnormalities in Branched-Chain α -Keto Acid Dehydrogenase Kinase-Deficient Mice, *Biochem. J. I. Pub.* BJ20060869 28Jul 2006.

Kubek, M.J., Yard, M., Lahiri, D.K., and Domb, A.J.: Characterization of Novel Intranasal Sustained-Release Nanoparticles for Delivery of Neuropeptides to the Brain. *Nanoparticles for Pharmaceutical Applications*. A. Domb, Y. Tabata, and N. V. Ravi Kumar, Eds., American Scientific Publishers, New York pp. 73-84 (2007)

Veronesi, M.C., Yard, M., Jackson, J., Lahiri, D.K, and Kubek, M.J.: Thyrotropin-Releasing Hormone (TRH) protects primary fetal rat (E17) cultured neurons against glutamate toxicity. *Brain Res.* 1128:79-85, 2007

Veronesi, M.C., Kubek, D.J. and Kubek, M.J.: Intranasal delivery of the thyrotropin-releasing hormone (TRH) analog attenuates seizures in the amygdala-kindled rat. *Epilepsia* doi:10.1111/j.1528-1167.2007.01218.x

Sen, A., Shannon, H.E. and Kubek, M.J.: Analysis of seizure EEG in kindled epileptic rats. *Comp. Math. Methods Medicine* 2007 (in press)

Veronesi, M.C., Kubek, D.J. and Kubek, M.J.: Isoflurane and halothane exacerbate seizures in amygdala-kindled rats during recovery. *Epilepsy Research* (in review)

C. Research Support

Ongoing or completed during the last 4 years:

NINDS, R13-NS049948-01 Neuropeptides and Epilepsy, 07/05/04 – 07/11/05, \$5,000 (Total) PI.

American Epilepsy Society: AES Workshop Grant: Neuropeptides and Epilepsy, 07/05/04 – 07/11/05, \$7,000 (Total) PI

Ortho-McNeil Pharmaceutical: Conference Grant CME support: Neuropeptides and Epilepsy, 07/05/04 – 07/11/05, \$4,000 (Total) PI

Bachem Corporation: Conference Grant: Neuropeptides and Epilepsy, 07/05/04 – 07/11/04, \$1,000 (Tot) PI

Durect Corporation: Conference Grant: Neuropeptides and Epilepsy, 07/05/04 – 07/11/04, \$750 (Tot) PI

UCB Pharmaceuticals: Conference Grant: Neuropeptides and Epilepsy, 6/27/06 – 7/01/06, \$3,500 (Tot) PI

The grants listed above are in support of satellite research symposia on Neuropeptides in Epilepsy as part of the Annual International and/or National Summer Neuropeptide Conference, Illana Gozas, Tel Aviv, Israel and Douglas Brenneman, Spring House, PA, Organizing Co-Chairs

BSF, United States Israel Bi-national Science Foundation- Long Acting Neurotransmitter Proteins for Tissue Specific Delivery to the Brain- For Treating Craniofacial Dysmorphias and Epilepsy, 10/01/99 - 09/30/03, \$150,765. (Total), U.S.A. Co-PI's, M.J. Kubek/K.E. Byrd; Israel PI, I. Ringel.

CURE: Citizens United for Research in Epilepsy: Intranasal Delivery of Sustained-Release Anticonvulsant Neuropeptide Nanoparticles in Seizure Therapy, 1/01/06 – 12/30/07, \$70,000. (Total), PI.

Pending

Epilepsy Research Foundation: New Therapy Grants: Intranasal delivery of sustained-release TRH nanoparticles in anticonvulsant therapy. 1/01/08 – 12/30/08, \$121,005. (Total), PI

CDMRP (DOD): New TBI Therapies Involving Novel Intranasal Delivery of Compounds. 3/01/08 – 3/30/2012, \$4M (Indirect), F. Leonessa, Co-PI, M.J. Kubek, Co-PI, \$1.1M (Total) subcontract.